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A fluorometric assay to determine antioxidant activity of both hydrophilic and lipophilic components in plant foods☆

Lei Li[a], C.-Y. Oliver Chen[b], Hye-Kyung Chun[c], Soo-Muk Cho[c], Ki-Moon Park[d], Yang Cha Lee-Kim[e], Jeffrey B. Blumberg[b], Robert M. Russell[b], Kyung-Jin Yeum[b,*]

[a]*Medical College of Qingdao University, Qingdao 266021, Shandong Province, China*
[b]*Jean Mayer USDA-Human Research Center on Aging at Tufts University, Tufts University, Boston, MA 02111, USA*
[c]*Rural Resources Development Institute, National Institute of Agricultural Science and Technology, Rural Development Administration, South Korea*
[d]*Department of Food Sciences and Biotechnology, Sunkyunkwan University, Suwon, 440-746, South Korea*
[e]*Department of Food and Nutrition, Yonsei University, 134 Shinchon-dong, Seodaemun-gu, Seoul, South Korea*

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Abstract

This study aimed to develop a fluorometric method to determine total antioxidant activity of plant foods. The antioxidant activities in plant foods were determined after extracting (1) hydrophilic components with acidified methanol (methanol:glacial acetate acid: water=50:3.7:46.3), (2) lipophilic components with methanol followed by tetrahydrofuran (THF), or (3) both hydrophilic and lipophilic components using sequential extraction of acidified methanol and THF together. Both the hydrophilic assay [using the hydrophilic radical initiator 2,2′-azobis-(2-amidinopropane)dihydrochloride (10 mmol/L) and hydrophilic probe 2,7-dichlorodihydrofluorescein (DCFH)] and the lipophilic assay [using the lipophilic radical initiator [2,2′-azobis (4-methoxiy-2,4-dimethylvaleronitrile), 2 mmol/L], and the lipophilic probe 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-undecanoic acid (C11-BODIPY 581/591) (BODIPY: 2 μmol/L)] were used to measure antioxidant activity. The inhibition of BODIPY oxidation was significantly increased ($P<.01$) when both the hydrophilic and lipophilic components were extracted using acidified methanol and organic solvent as compared to those extracted by organic solvent alone. In addition, the rate of DCFH oxidation was significantly delayed ($P<.05$) when both components coexisted compared to DCFH oxidation of the hydrophilic component alone. The combination of lipophilic and hydrophilic components in these plant foods showed significantly greater antioxidant activity than that of either hydrophilic or lipophilic component alone. Thus, both hydrophilic and lipophilic components in plant foods and their interactions should be considered when determining their antioxidant activity.

Keywords: Azo-initiator; Vegetables; Fluorescence; Total antioxidant activity; DCFH; BODIPY

1. Introduction

Epidemiologic studies suggest that diets rich in fruits and vegetables are protective against degenerative diseases such as cardiovascular disease and some forms of cancer [1,2]. Beneficial effects of these plant foods have been attributed, in part, to their antioxidant constituents [3,4]. A wide range of methods has been described in the literature for assessing the antioxidant activity of fruits and vegetables [5–7]. However, most of these methods focus on determining only the hydrophilic antioxidant components such as phenols and vitamin C.

Some plant foods such as dark green leafy vegetables are rich not only in water-soluble but also fat-soluble antioxidants including tocopherols and carotenoids. In our previous study, 95 plant food samples collected in Korea were analyzed, among which *Angelica keiskei* and *Perilla*, dark

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* Corresponding author. Tel.: +1 617 556 3204; fax: +1 617 556 3344.
E-mail address: kyungjin.yeum@tufts.edu (K.-J. Yeum).

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green leafy vegetables consumed widely in Asia and black beans are rich in fat-soluble antioxidants, especially carotenoids and tocopherols [8]. Thus, we have focused our attention on developing a method to measure the antioxidant activity of the lipophilic or hydrophilic components as well as their combined activity in the plant foods collected in Korea. Three different methods were applied to extract the hydrophilic and lipophilic antioxidant components and a combination of both. Antioxidant activity was determined by both (1) a lipophilic assay using 2,2′-azobis (4-methoxiy-2,4-dimethylvaleronitrile) (MeO-AMVN), as a lipid soluble radical initiator, and 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-undecanoic acid (C11-BODIPY 581/591) (BODIPY) as a lipophilic fluorescence probe and (2) a hydrophilic assay using 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH) as the radical generator and 2′,7′-dichlorodihydrofluorescein (DCFH) as the probe.

2. Materials and methods

2.1. Chemicals

Radical initiators AAPH and MeO-AMVN were obtained from Wako Chemicals (Richmond, VA, USA). The fatty acid analogue BODIPY and 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) were obtained from Molecular Probes (Eugene, OR, USA). Methanol and tetrahydrofuran (THF) were purchased from Fisher Scientific (Pittsburgh, PA, USA). All reagents used in this study were of analytical grade. Phosphatidylcholine was purchased from Sigma (St. Louis, MO, USA).

2.2. Sample preparation

All plant food samples were harvested and processed in Korea. Sample processing was conducted under dim light on the day of collection. After thorough cleaning using tap water, the edible portions of foods, without peels, seeds and stalks, were lyophilized for 70 h (Ilshin-lab, PVTFD10A, Korea). The lyophilized samples were ground to powder (Samsung, SM-550, Korea) and aliquots of 100 mg were placed in brown glass vials. Each vial was filled with nitrogen gas and stored at −80°C. Samples were hand-carried in dry ice-packs to the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University (Boston, MA, USA) within 2 months of collection and stored at −80°C until analysis.

2.3. Extraction of phytonutrients of plant-based foods

2.3.1. Fat-soluble components

Fat-soluble components including carotenoids and tocopherols were extracted as previously reported, with minor modifications [9]. Briefly, 200 mg lyophilized samples were incubated with methanol for 2 h at room temperature, followed by 4 sequential extractions with 10 ml THF. Two ml extract was dried under nitrogen gas, dissolved in 0.5 ml Tween-60/chloroform (3 mg/ml), dried under nitrogen gas, and reconstituted in 1 ml PBS (50 nmol/L, pH 7.4) for further testing.

2.3.2. Water-soluble components

Two hundred milligrams of lyophilized sample was used to extract the water-soluble components. Samples were incubated with 10 ml acidified methanol (methanol: glacial acetate acid:water=50:3.7:46.3) at room temperature for 30 min in a shaking water bath (Labline/Dubnoff incu-shaker 3375), followed by centrifugation at 800 *g* for 10 min; the supernatant was dried under nitrogen gas and dissolved in 1 ml phosphate-buffered saline (PBS) (50 nmol/L, pH 7.4) solution.

2.3.3. Fat- and water-soluble components together

Two hundred milligrams of lyophilized sample was used to extract the water- and fat-soluble components. Samples were incubated with 10 ml acidified methanol (methanol: glacial acetate acid:water=50:3.7:46.3) at room temperature for 30 min in a shaking water bath (Labline/Dubnoff incu-shaker 3375), followed by centrifugation at 800 *g* for 10 min; the supernatant of 10 ml was dried under nitrogen gas, dissolved in 2 ml PBS (50 nmol/L, pH 7.4) and set aside. The solid residue of the acidified methanol extraction was further extracted 4 times with 10 ml THF. Two milliliters of THF extract were dried under nitrogen gas, dissolved in 0.5 ml Tween-60/chloroform (3 mg/ml), dried under nitrogen gas, and resuspended in 1ml PBS solution, which was set aside. The extraction procedures for fat-soluble, water-soluble and combined components are presented in Fig. 1.

2.4. Analysis of antioxidant activity in plant-based foods

2.4.1. Lipophilic analysis using MeO-AMVN and BODIPY

Antioxidant activity was measured fluorometrically using MeO-AMVN and BODIPY. BODIPY was dissolved in dimethyl sulfoxide, aliquoted, and stored at −80°C. For BODIPY incorporation into reaction mixture, 25 μl BODIPY stock solution (2 mmol/L) was first diluted 100-fold with PBS. An aliquot of 100 μl (20 μmol/L) was added to appropriate volumes of samples, vortexed for 20 sec at the lowest speed, and incubated for 10 min at 37°C. Then, 15 μl MeO-AMVN was added to the sample and diluted to a final volume of 1 ml with PBS, yielding BODIPY and MeO-AMVN at final concentration of 2 μmol/L and 2 mmol/L, respectively.

Aliquots of 200 μl reaction mixture were transferred to a 96-microwell plate, and the oxidation kinetics were monitored by measuring the florescence of the oxidation product of BODIPY. The excitation wavelength (λex) was set at 485 nm and the emission wavelength (λem) was set at 535 nm [10]. The results are expressed as the percentage of inhibition of DCFH and BODIPY with respect to that of a control sample.

The control sample was phosphatidylcholine (PC) liposomes prepared as follows: soybean phosphatidylcholine was dissolved in chloroform (0.25 mg/L) and evaporated

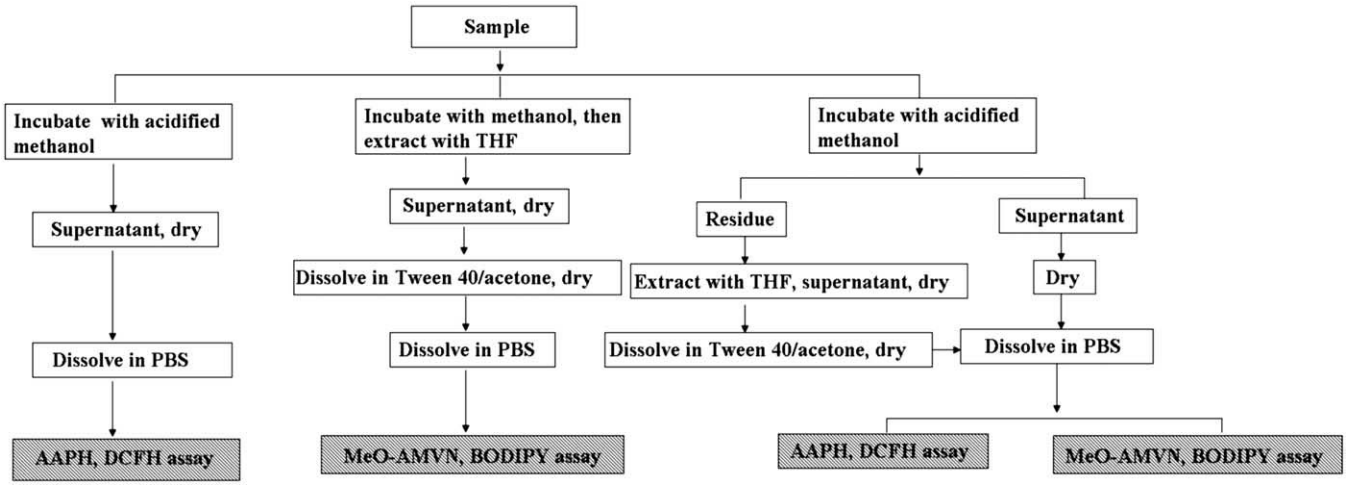

Fig. 1. The flowchart of experimental procedure.

slowly under a stream of nitrogen gas. A thin lipid film was formed on the walls by rolling the vial. The vial was then maintained under a nitrogen stream for an additional 20 min to eliminate residual solvent. The lipid film was then rehydrated with PBS (40 mmol/L, pH 7.4) to a concentration of 3 mg/ml phospholipid and treated in a water bath sonicator (four cycles of 10 s each), aliquoted to 500 μl each and stored at −80°C.

2.4.2. Hydrophilic analysis using AAPH and DCFH

Antioxidant activity was measured fluorometrically using AAPH and DCFH. DCFH-DA stock solution (1 mmol/L) was prepared in EtOH, stored at −20°C and used within 2 months. DCFH was prepared from DCFH-DA by basic hydrolysis. Briefly, 500 μl of DCFH-DA stock solution (1 mmol/L) was mixed with 2 ml NaOH (0.01 N at 4°C) for 20 min, protected from light and then neutralized with 2 ml HCl (0.01 N), diluted with PBS to a final concentration of 10 μmol/L and stored in ice for <8 h (working solution). A 100-μl aliquot of DCFH (10 μmol/L) was added to appropriate sample volumes followed by 20 μl AAPH (10 mmol/L) and then diluted to a final volume of 1 ml with PBS. Oxidation was measured by monitoring the 2-electron oxidation of DCFH to the highly fluorescent 2′,7′-dichlorofluorescein. The excitation wavelength (λex) was set at 485 nm and emission (λem) at 535 nm [10].The results are expressed as the percentage of inhibition of DCFH oxidation with respect to that of a control sample. The control was PC liposomes, the same as that in lipophilic assay using MeO-AMVN and BODIPY.

2.5. Analysis of phytonutrients in plant-based foods

2.5.1. Analysis of carotenoids and tocopherols

Carotenoids and tocopherols were quantified using high-performance liquid chromatography (HPLC) with a C30 column (3.0 μm, 3.0×150 mm, ProntoSIL, PA, USA) and monitored at 455 nm and 292 mm, respectively, with a Waters 2996 photodiode array detector (Milford, MA, USA). All the carotenoids and tocopherols were quantified by determining peak areas under the curve calibrated against known amounts of standards. Each peak was confirmed by the retention time and characteristic spectra of the standards. The lower limits of detection were 0.2 pmol for carotenoids and 2 pmol for tocopherols. The interassay coefficient of variation (CV) was 4% (n=25) and the intraassay CV was 4% (n=9).

2.5.2. Analysis of ascorbic acid and polyphenols

Ascorbic acid was extracted as reported with minor modification [11]. One hundred milligrams of lyophilized sample was incubated with 5 ml perchloric acid for 2 h, vortexed and centrifuged. The supernatant was injected into an HPLC system with Millennium 32 software (Waters Associates) and a BAS EC-5 electrochemical detector with amperometric detection (Bioanalytical Systems).

Polyphenols in 100 mg lyophilized sample were sequentially extracted twice with acidified methanol (methanol: glacial acetate acid: water=50:3.7:46.3) over a 16-h period. The combined extract was dried under nitrogen gas. The dry residue was reconstituted with water. The total phenol content was analyzed with Folin–Ciocalteu reagent according to the method of Singleton [12]. Absorbance was determined at 725 nm using a Shimadzu UV1601 spectrophotometer (Tokyo, Japan). The total phenol content was calculated based on a standard curve established with gallic acid. The final results were expressed as mg gallic acid equivalents per gram of dry weight.

2.6. Statistical analysis

The data are expressed as mean±S.D. Tukey's test was performed to identify differences among groups. Because the data were not normally distributed, a Spearman rank-order correlation analysis was conducted to determine correlations between the various phytonutrients and oxidation inhibition of BODIPY or DCFH. All statistical analyses were determined using SAS (SAS version 9.1, SAS Institute

Inc, Cary, NC, USA). Statistical significance was defined as $P<.05$.

3. Results

3.1. Phytonutrient concentrations in plant foods

Table 1 shows the mean phytonutrient contents of plant foods. *Angelica keiskei* had the highest content of lutein and β-carotene at 212 and 106.28 μg/g, respectively, followed by *Perilla* leaves (147.38 and 87.62 μg/g) and pumpkin young leaves (121.27 and 53.96 μg/g). Black beans contain γ-tocopherol at 84.21 μg/g, which is much higher than that in green leafy vegetables. For ascorbic acid, *Angelica keiskei* had the highest content (20.08 μg/g).

3.2. Effect of the amount of extracts (volume) on the oxidation of plant-based foods: lipophilic assay using MeO-AMVN and BODIPY

To determine the dose-dependent antioxidant activity of plant foods, experiments employing different volumes of samples were conducted. The extracts of 100, 200, 300 and 400 μl were used for analyzing the antioxidant activity of plant foods with the lipophilic assay using MeO-AMVN and BODIPY. With increasing extraction volume, BODIPY oxidation decreased ($P<.05$) (Fig. 2). The oxidation kinetics of the 200-μl extract showed an optimal kinetic curve of BODIPY oxidation, so this volume was used in subsequent experiments.

3.3. Effect of extraction methods on oxidation kinetics of plant foods

Antioxidant activities of plant foods were determined using either the fat-soluble component alone or both the water- and the fat-soluble components together. Fig. 3 shows that the oxidation of BODIPY in the control solution, which did not contain any antioxidant, was considerably more rapid than that of fat-soluble or combined extracts. Furthermore, the oxidation of BODIPY in the water- and fat-soluble extracts together showed a delay as compared to that of fat-soluble extract alone.

Table 2 shows antioxidant activities of green leafy vegetables and black beans determined by the lipophilic and hydrophilic assays. The oxidation of BODIPY was inhibited significantly by both the combined water- and fat-soluble extracts of plant foods as compared to the oxidation when using fat-soluble extracts alone ($P<.05$).The inhibition via the combined water- and fat-soluble extracts on the DCFH oxidation was also significantly higher than that using water-soluble extracts alone ($P<.05$). The inhibition of DCFH oxidation by the combined water- and fat-soluble extracts (87.6%) from pak-choi was almost twice as high of that when using the water-soluble extract alone (38.7%).

3.4. Correlation between phytonutrients and antioxidant activity

There was a significant correlation between inhibition of BODIPY oxidation and total carotenoid and tocopherol concentrations in the plant-based foods tested ($n=10$, $r=.675$, $P<.05$) (Fig. 4A). However, no significant correlation was found between the total carotenoid and tocopherol concentration and inhibition of BODIPY oxidation when the water- and fat-soluble extracts were used to determine antioxidant activity ($n=10$, $r=0.328$, $P=.355$) (Fig. 4B). There was a significant correlation between the inhibition of DCFH oxidation with total phenols ($n=10$, $r=0.866$, $P<.01$) (Fig. 5A). On the other hand, there was no significant correlation (Fig. 5B) between total phenols and inhibition of DCFH oxidation when the water- and fat-soluble extracts were used to determine antioxidant activity ($n=10$, $r=0.454$, $P=.188$).

4. Discussion

In food analysis, individual phytochemicals can be identified and measured. Even though the antioxidant profiles of plant foods are informative, this approach is

Table 1
Phytonutrient concentrations of green leafy vegetables and black beans (mean±SD)

Sample	Latin name	Lutein	*trans*-β-Carotene	9-*cis*-β-Carotene	α-Tocopherol	γ-Tocopherol	Total phenols (mg/g)	Ascorbic acid (μg/g)
Angels' plant	*A. keiskei*	212.00±8.20	106.28±5.81	18.08±1.41	–	–	9.77	20.08
Black beans	*Glycine max* Merr.	11.86±1.12	–	–	10.19±0.20	84.21±3.49	3.81	0.24
Pak-choi	*Brassica campestris*	3.11±0.20	24.89±1.22	3.89±0.22	14.11±2.10	–	7.36	4.43
Cauliflower	*Brassica oleracea botrytis*	0.22±0.00	0.11±0.00		6.28±0.98	1.54±0.12	8.40	6.48
Kale	*B. oleracea sabellica*	53.78±7.89	30.78±4.71	6.90±0.99	35.05±5.20	–	11.02	3.10
India spinach	*Spinacia oleraceae*	89.59±4.66	35.25±0.76	6.40±0.33	22.02±2.60	–	12.05	2.67
Chicory	*Cichorium intybus*	37.62±1.59	23.38±0.85	3.61±0.21	0.32±0.00	–	10.89	0.23
Young radish	*Raphanus sativus* L.	44.63±3.80	31.16±3.04	4.89±0.43	0.65±0.00	–	10.65	3.02
Pumpkin young leaves	*Cucurbita maxima*	121.27±8.26	53.96±4.30	8.03±0.79	60.97±5.25	–	15.62	3.92
Perilla leaves	*Perilla frutescens*	147.38±9.52	87.62±0.24	12.02±0.24	38.93±3.02	–	12.08	0.27

Carotenoids and tocopherols were analyzed in triplicate. Total phenols and ascorbic acid were analyzed in duplicate.
The unit of carotenoids and tocopherol was μg/g dry weight.

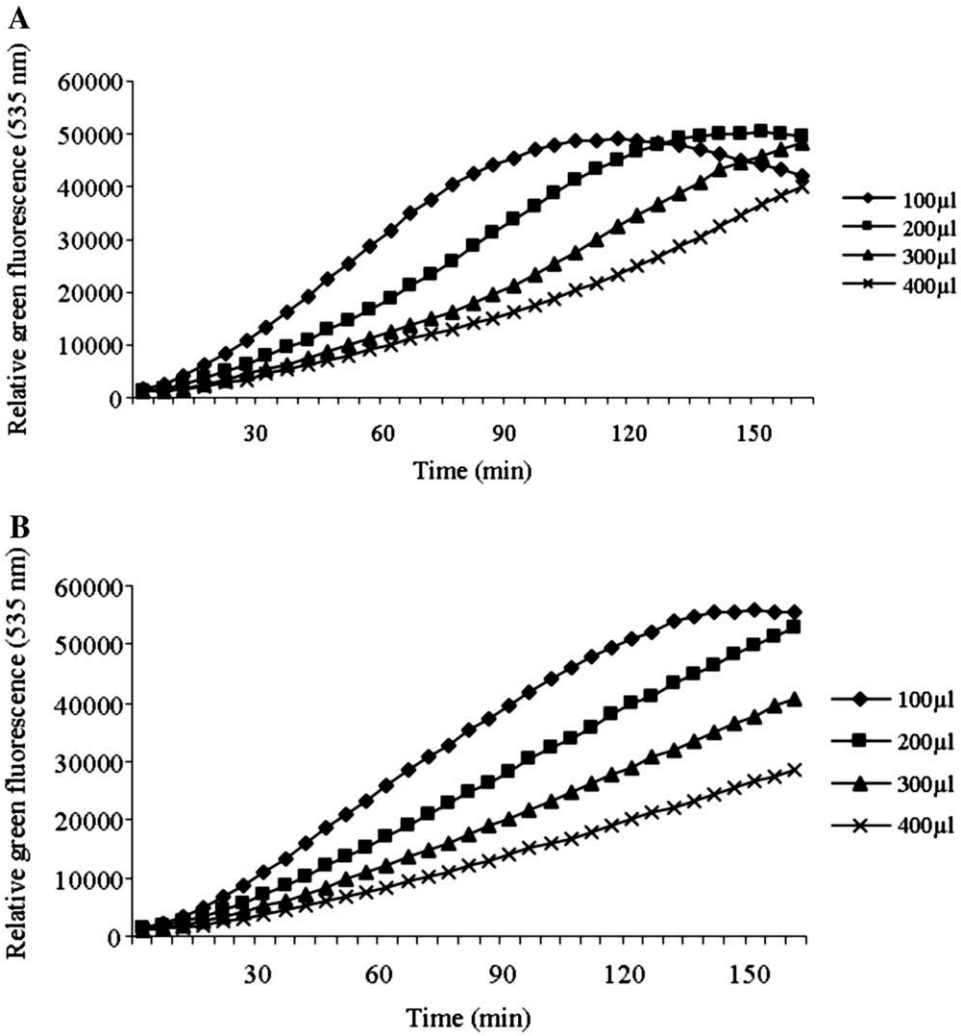

Fig. 2. Effects of the amount (volume) of spirulina (A) and yellow pepper (B) extracts on the BODIPY oxidation kinetics. Oxidation was initiated with MeO-AMVN and monitored by BODIPY. (Spirulina and pepper were extracted with THF.)

time-consuming and requires a variety of analytical techniques for the measurement of both aqueous and lipid antioxidants. This approach also lacks information on the combined effect of hydrophilic and lipophilic antioxidants. Therefore, it is desirable to establish a method that can measure the overall antioxidant activity, including interactions directly from vegetable extracts. In this study, we extracted both water- and fat-soluble antioxidants to determine their combined antioxidant activity. Thirty minutes of incubation in vigorous shaking water bath was used for extraction of water-soluble components in selected plant foods for antioxidant activity determination. The efficiency of total phenols extraction by this modified method was 61±12% (n=6) as compared to that of traditional method using 16 h sequential extraction.

Recently, several methods have been developed to measure "total antioxidant activity" [13], "total antioxidant capacity [14,15] or "total antioxidant potential" [16,17], among which, Trolox equivalent antioxidant capacity (TEAC) [18], total radical absorption potentials [19], ferric-reducing/antioxidant power [20] and oxygen radical absorption capacity (ORAC) assays [21] are widely used. Both ORAC and TEAC assays measure the inhibition of the added free radicals. The ORAC assay uses AAPH as a free radical generating system, B-phycoerythrin or R-phycoerythrin as a sensitive target probe of free radical attack and an area-under-curve technique for the quantification [22,23]. AAPH undergoes spontaneous decomposition and produces peroxyl radicals, with a rate primarily determined by temperature [24]. The ORAC assay has been used by different laboratories [25,26] and has provided limited information regarding the antioxidant capacity of various plant foods because of ineffective response to fat-soluble antioxidants. As a variation of this assay, Huang et al [27] reported an ORAC assay for lipophilic antioxidants, which measures vitamin E and other common lipophilic phenolic antioxidants. The antioxidant activity of water-soluble components cannot be determined in this assay since this

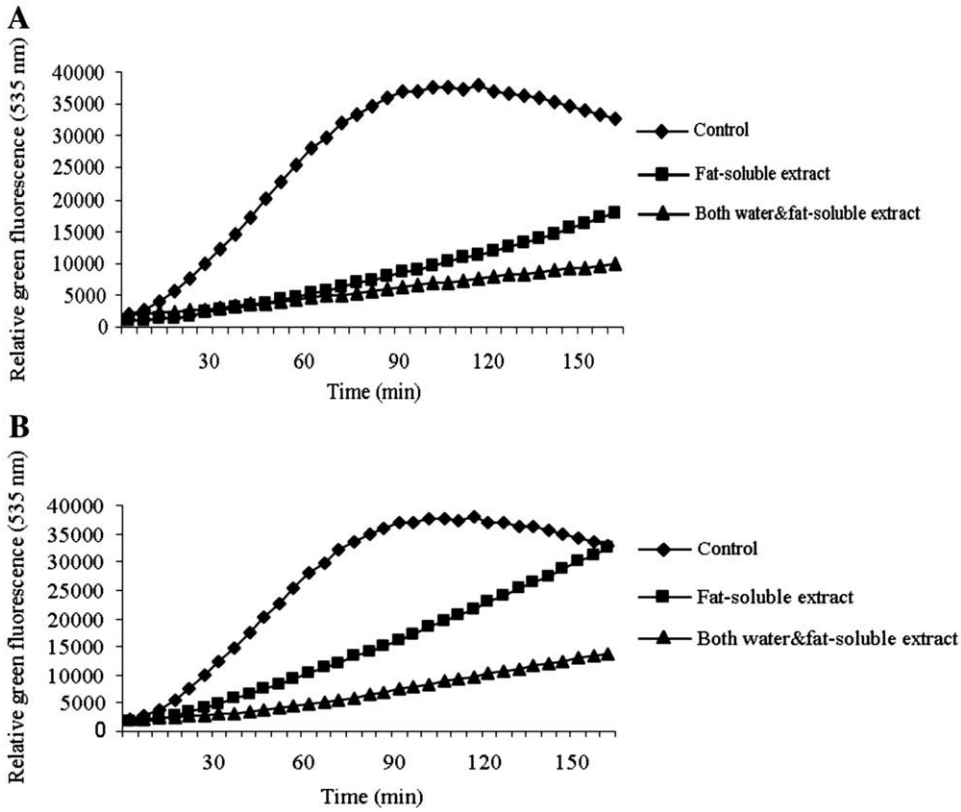

Fig. 3. Effect of extraction methods of *A. keiskei* (A) and spinach (B) on the oxidation kinetics of BODIPY. Oxidation was initiated with MeO-AMVN and monitored by BODIPY. Fat-soluble components were extracted with THF; both water- and fat-soluble components were extracted with acidified methanol and THF. PC liposomes were used as the control.

assay uses acetone for lipophilic component extraction. Thus, interaction between lipophilic and hydrophilic components cannot be measured in this assay.

As reported, most of assays based on the use of a hydrophilic radical inducer, AAPH, and a hydrophilic probe primarily measure the antioxidant capacity of hydrophilic components of plant foods [28]. In this study, AAPH and DCFH were also selected as the radical inducer and probe, respectively, to determine the antioxidant activity of water-soluble components. However, we used this radical initiator and probe in combination with hydrophilic and lipophilic components of plant foods and found that the protection of DCFH oxidation by the combined water- and fat-soluble extracts was much higher than that of water-soluble extract alone. It is interesting to note that antioxidant activity of combined water- and fat-soluble antioxidants was larger than water-soluble antioxidants alone when fat-soluble antioxidants were not responsive in this assay. Thus, this result

Table 2
Antioxidant activity of green leafy vegetables and black beans determined by the hydrophilic and lipophilic assays (mean±S.D.)

Sample	Latin name	Inhibition of DCFH oxidation (%)		Inhibition of BODIPY oxidation (%)	
		Water-soluble	Water- and fat-soluble	Fat-soluble	Water- and fat-soluble
Angelica keiskei	*A. keiskei*	79.60±2.12	90.56±3.20 *	61.35±3.51	80.03±1.40 *
Black beans	*Glycine max Merr.*	49.49±3.58	76.86±1.03 *	52.05±2.86	61.42±1.30
Pak-choi	*B. campestris*	38.67±1.93	87.58±2.96 *	54.27±2.55	63.52±1.67
Cauliflower	*Brassica oleracea botrytis*	34.63±2.47	86.61±1.25 *	36.54±1.84	82.06±1.70 *
Kale	*Brassica oleracea sabellica*	67.54±4.44	86.46±3.75 *	63.43±3.89	80.34±1.89 *
Spinach	*S. oleraceae*	70.57±2.36	85.63±3.20 *	67.10±2.95	75.70±2.23
Chicory	*C. intybus*	65.66±3.12	90.12±2.55 *	63.93±2.85	80.37±3.48 *
Young radish	*Raphanus sativus* L.	49.62±4.68	85.14±2.41 *	65.92±3.59	71.48±1.87
Pumpkin young leaves	*Cucurbita maxima*	69.63±4.71	88.17±2.07 *	78.81±1.79	75.64±1.29
Perilla leaves	*P. frutescens*	78.93±4.36	90.63±2.32 *	84.02±2.91	82.18±2.03

Samples were analyzed in triplicate.

* $P<.05$ as compared with water soluble or fat soluble extract alone.

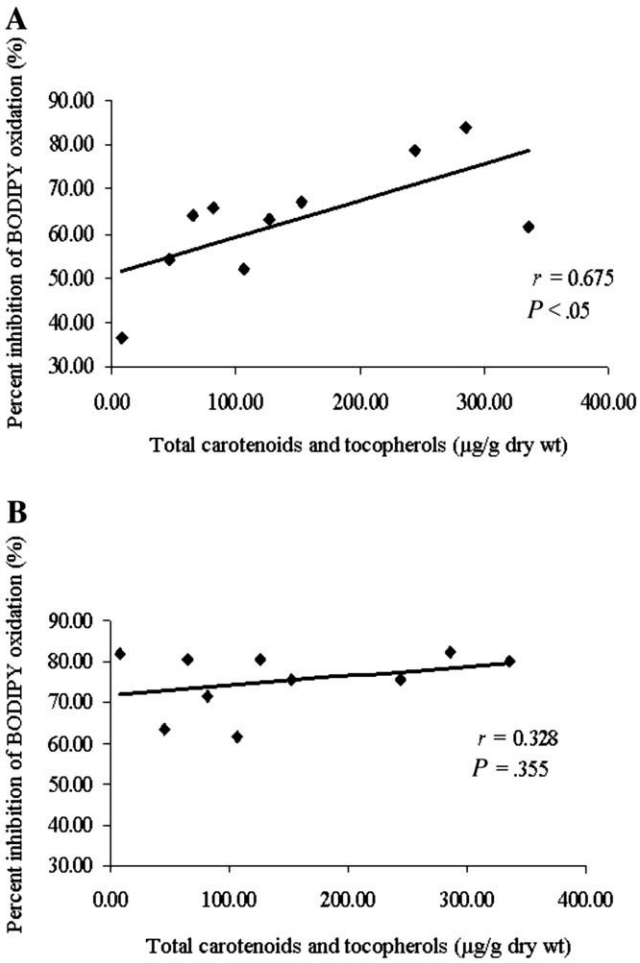

Fig. 4. Correlation between total carotenoids and tocopherol and inhibition of BODIPY oxidation by the fat-soluble components alone (A) or water- and fat-soluble combined components (B) (n=10). A Spearman rank-order correlation analysis was conducted. Correlation coefficients (r) and the significance values (P) are indicated. (Fat-soluble components were extracted with THF; both water- and fat-soluble components were extracted with acidified methanol and THF.)

suggests that water- and fat-soluble antioxidants work to protect DCFH against peroxyl radicals either in an additive or synergistic manner.

In an effort to understand the biological significance of lipophilic antioxidants, we have investigated the antioxidant activity of fat-soluble components in plant foods [8]. Also, we focused our attention on the antioxidant activity of the hydrophilic and lipophilic components together. Our results show that the inhibition of BODIPY oxidation by the combined water- and fat-soluble extracts was much higher than that of fat-soluble antioxidant alone, indicating that both water- and fat-soluble antioxidants contribute to the significant protection of BODIPY oxidation. Water-soluble extract did not show any effect on BODIPY oxidation because of the lipophilicity of BODIPY and MeO-AMVN.

As previously reported [29], new approaches to define the "true" total antioxidant capacity in the circulation should reflect the antioxidant network interactions between water- and fat-soluble antioxidants in vivo. However, to determine the antioxidant capacity of a plant food in vivo, one would also need to take into account the bioavailability of the various antioxidants from the food matrix.

Phytonutrients such as carotenoids, tocopherols, ascorbic acid and total phenols were analyzed to understand their contribution to the antioxidant activity of plant foods. As previously reported [8], water- and fat-soluble extracts show significant correlation with ORAC and lipophilic antioxidant performance, respectively. On the other hand, no correlation was found between fat-soluble components in these plant foods and BODIPY oxidation when the combined water- and fat-soluble extracts were used to determine antioxidant activity. Thus, it is clear that fat-soluble antioxidants as well as water-soluble antioxidants participate in protecting the oxidation of BODIPY. Similarly, the significant correlation between water-soluble phenols and inhibition of DCFH

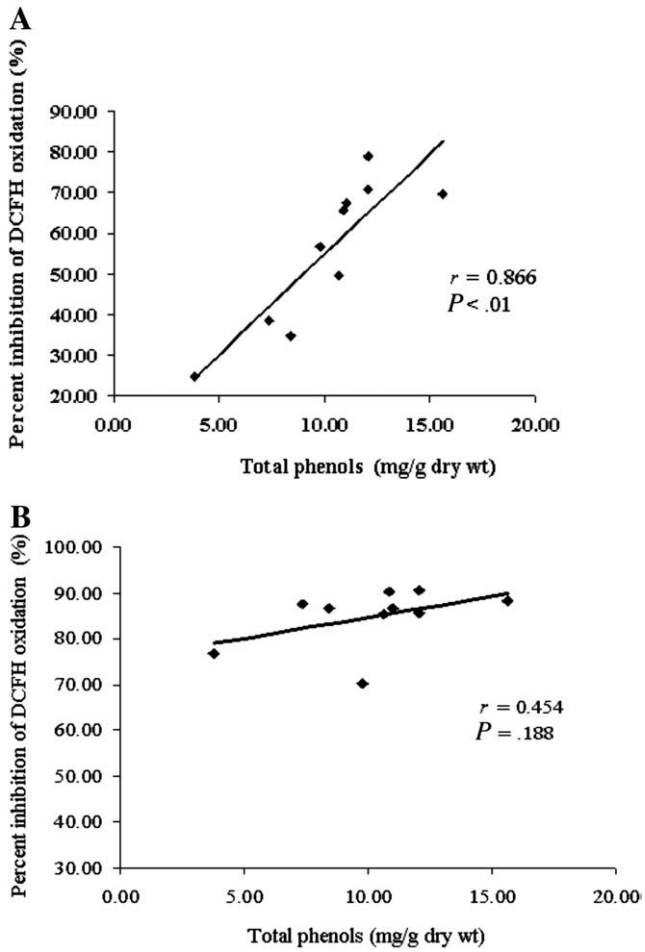

Fig. 5. Correlation between total phenols and inhibition of DCFH oxidation by the water-soluble components (A) or by the water- and fat-soluble combined components (B) (n=10). A Spearman rank-order correlation analysis was conducted. Correlation coefficients (r) and the significance values (P) are indicated. *Water-soluble components were extracted with acidified methanol; both water- and fat-soluble components were extracted with acidified methanol and THF.

oxidation by the water-soluble extract no longer existed when the combined water- and fat-soluble extracts were used to determine antioxidant activity. Total phenols are the major antioxidants in water-soluble extract and play an important role in the inhibition of DCFH oxidation. On the other hand, when the combined water- and fat-soluble extracts were used, not only water-soluble antioxidants such as total phenols but also fat-soluble antioxidants such as carotenoids exerted a protective effect on the DCFH oxidation. When both water- and fat-soluble antioxidants coexist, both antioxidants participate in the protection of oxidation of either hydrophilic probe, DCFH, or lipophilic probe, BODIPY. Therefore, it is not surprising to lose tight correlations, as was shown between water-soluble antioxidants and hydrophilic DCFH oxidation or between fat-soluble antioxidants and lipophilic BODIPY oxidation.

Our results indicate that the overall antioxidant activities in plant foods are significantly increased when both their water- and fat-soluble components coexist as compared to when either the water-soluble or fat-soluble components are tested individually. Therefore, determination of the antioxidant activity of plant foods should consider both their hydrophilic and lipophilic components using a proper assay, as is reported in the current study.

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